

What is Claimed is:

1. A method for determining tumor maintenance function of a gene comprising the steps of:
 - 5 (a) producing a mammalian host cell comprising (1) an oncogene expression construct, (2) a genetic mutation, conditional gene knockout or an RNA reagent that causes reduced expression or activity of a tumor suppressor gene, and (3) a coding sequence for an RNA interference (RNAi) molecule against the gene, said coding sequence operably linked to an inducible promoter;
 - 10 (b) introducing the host cell into an animal at a desired developmental stage, wherein the host cell develops into a tumor; and
 - (c) inducing expression of the RNAi molecule, wherein regression of the tumor indicates that the gene is involved in tumor maintenance.
- 15 2. The method of claim 1, wherein the tumor maintenance function is selected from the group consisting of the function required to sustain tumor size, the function required to maintain tumor viability, and the function required to facilitate tumor growth.
- 20 3. The method of claim 1, wherein tumor regression is indicated by apoptosis of tumor cells, reduction in tumor size, collapse of tumor vasculature, or reversion of tumorigenic phenotype of tumor cells.
- 25 4. A method for determining tumor maintenance function of a gene comprising the steps of:
 - (a) producing a mammalian host cell comprising (1) an oncogene expression construct, (2) a genetic mutation, conditional gene knockout or an RNA reagent that causes reduced expression or activity of a tumor suppressor gene, and
 - 30 (3) a coding sequence for an RNAi molecule against the gene, said coding sequence operably linked to an inducible promoter;
 - (b) inducing expression of the RNAi molecule; and

- 16 -

(c) comparing viability and/or proliferation of cells expressing the RNAi molecule and cells not expressing the RNAi molecule,

wherein decreased cell viability and/or proliferation of cells expressing the RNAi molecule, compared to cell viability and/or proliferation of cells not expressing the RNAi molecule, indicates that the gene is involved in tumor maintenance.

5 5. The method of claim 1 or 4, wherein the RNAi molecule is about 19 to about 29 basepairs in length for the double-stranded portion of the molecule.

 6. The method of claim 1 or 4, wherein the RNAi molecule is a hairpin RNA having about 19 to about 29 basepairs in the stem portion of the molecule, and about 4 to about 34 nucleotides in the loop portion of the molecule.

15 7. The method of claim 1 or 4, wherein the oncogene is a dominant acting form of H-ras, K-ras, N-ras, c-myc, n-myc, EGFR, MDM2, BDNF, her2/neu/erb-B2, TGFb, RhoC, VEGF-C, AKT, abl, src, raf, fos, or b-catenin.

20 8. The method of claim 1 or 4, wherein the tumor suppressor gene is selected from the group consisting of Ink4a/arf, pten, rb, and p53.

 9. A vector for expressing an RNAi molecule comprising an RNA polymerase transcription unit, and either (1) sense or antisense sequences constituting the RNAi molecule, wherein the sense and antisense sequences are transcribed by individual promoters; or (2) a sequence encoding the RNAi molecule, wherein the sequence encodes a fold-back stem-loop structure (hairpin) that give rise to dsRNAs after intracellular processing.

30 10. The vector of claim 9, wherein the transcription unit is an RNA polymerase III transcription unit.

- 17 -

11. The vector of claim 10, wherein the transcription unit is a U6 transcription unit.

5 12. The vector of claim 9 further comprising an inducible transcription-regulatory element.

13. The vector of claim 9, wherein the vector is a lentiviral vector.

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14. A vector comprising (1) a Gateway cassette containing a U6-based transcription unit for expressing a gene-specific RNAi molecule, wherein the transcription unit comprises a promoter region, and wherein a Lac operator is inserted into the promoter region; and (2) a coding sequence for a Lac repressor and a coding sequence for a fluorescent protein wherein the coding sequences are separated by an IRES and wherein the coding sequences are under the control of a constitutive promoter.

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15. A lentiviral vector comprising
20 (1) a Gateway cassette comprising a U6-based transcription unit for expressing a gene-specific RNAi molecule, wherein the transcription unit comprises a promoter region, and wherein a Lac operator sequence is inserted into the promoter region;

(2) a U6-based transcription unit for expressing a tumor suppressor gene-specific RNAi (ts-RNAi) molecule;

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(3) a coding sequence for a Lac repressor and a coding sequence for a fluorescent protein wherein the coding sequences are separated by an IRES and wherein the coding sequences are under the control of a constitutive promoter; and

(4) an oncogene under the control of a constitutive promoter.

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16. The vector of claim 14 or 15, wherein the oncogene is a dominant acting form of H-ras, K-ras, N-ras, c-myc, n-myc, EGFR, MDM2,

- 18 -

BDNF, her2/neu/erb-B2, TGFb, RhoC, VEGF-C, AKT, abl, src, raf, fos, or b-catenin.

17. The vector of claim 14 or 15, wherein the tumor suppressor
5 gene is selected from the group consisting of Ink4a/arf, pten, rb, and p53.

18. The vector of claim 14 or 15, wherein the fluorescent protein
is selected from the group consisting of green fluorescent protein, blue fluorescent
protein, yellow fluorescent protein, and luciferase.

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19. The vector of claim 14 or 15, wherein the constitutive
promoter is a tissue-specific promoter.

20. A host cell comprising the vector of claim 4 or 15.

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21. A host cell comprising the vector of claim 14, wherein the
host cell further comprises an activating mutation in an oncogene and/or an
inactivating mutation in a tumor suppressor gene.

22. A host cell comprising (1) an oncogene, (2) a ts-RNAi
20 molecule, and (3) an inducible gene-specific RNAi molecule, wherein the
oncogene, ts-RNAi molecule, and inducible gene-specific RNAi molecule are
cloned into one or two lentiviral vectors.

23. A host cell comprising:
(a) a first vector comprising (1) a U6-based transcription unit for
expressing a ts-RNAi molecule, and (2) a coding sequence for a Lac repressor and
a coding sequence for a fluorescent protein wherein the coding sequences are
separated by an IRES and wherein the coding sequences are under the control of a
25 constitutive promoter; and

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(b) a second vector comprising (1) a Gateway cassette comprising a
U6-based transcription unit for expressing a gene-specific RNAi molecule,

- 19 -

wherein the transcription unit comprises a promoter region, and wherein a Lac operator is inserted into the promoter region; and (2) an oncogene under the control of a constitutive promoter.

5 24. A host cell comprising

 (a) a first vector comprising an rtTA-coding sequence under the control of a constitutive promoter; and (2) a coding sequence for a Lac repressor and a coding sequence for a fluorescent protein wherein the coding sequences are separated by an IRES and wherein the coding sequences are under the control of a
10 constitutive promoter; and

 (b) a second vector comprising (1) a U6-based transcriptional unit for expressing a ts-RNAi molecule; (2) a Gateway cassette comprising a U6-based transcription unit for expressing a gene-specific RNAi molecule, wherein the transcription unit comprises a promoter region, and wherein a Lac operator is
15 inserted into the promoter region; and (3) an oncogene under the transcriptional control of the rtTA and tetracycline.

 25. The host cell according to claims 23 or 24 wherein the first and/or the second vector is a lentiviral vector.

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 26. A chimeric or transgenic non-human animal comprising the host cell of any one of claims 20-25.

 27. A method of determining toxicity of inhibiting the
25 expression or activity of a gene in an animal comprising the steps of

 (a) producing an animal, wherein the animal comprises a cell comprising a coding sequence for an RNAi molecule against the gene, said coding sequence operably linked to an inducible promoter; and

 (b) inducing expression of the RNAi molecule, wherein a decrease
30 in cell viability is indicative of toxicity.

- 20 -

28. The method of claim 27, wherein the RNAi molecule is expressed in a specific tissue.